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STUDIES ON BRAIN METABOLISM OF BIOGENIC AMINES

U. SCHACHT, M. LEVEN & G. BÄCKER
Department of Biochemistry, Hoechst AG, Postfach 80 03 20, D-6230 Frankfurt/Main 80

1 *In vitro* models (synaptosones, electrically stimulated brain slices and monoamine oxidase (MAO) preparations have been used to identify the sites of action of nomifensine on brain monoamine metabolism.

2 Nomifensine potentiates neurotransmission in noradrenergic and dopaminergic synapses by blocking catecholamine uptake.

3 Nomifensine does not inhibit MAO and does not enhance the release of biogenic amines.

4 Studies on electrically stimulated brain slices suggest that nomifensine does not directly stimulate dopamine (DA) nor noradrenaline (NA) receptor sites.

5 5-Hydroxytryptamine (5-HT) uptake is not inhibited at 'therapeutic' concentrations of nomifensine.

6 Nomifensine differs from the tricyclic antidepressants by virtue of its effect on the dopaminergic system.

Introduction

Antidepressant activity is assumed to result from the enhancement of central monoaminergic function, which is regarded as deficient in depressed patients (Schildkraut, 1974; Baldessarini, 1975). According to widely accepted hypotheses, the therapeutic efficacy of the known antidepressant drugs is based on a blockage of the inactivation mechanisms of the biogenic amines NA, DA, and/or 5-HT. The re-uptake of the monoamines into the presynaptic nerve endings by way of a specific membrane-bound carrier system, and their enzymatic degradation, mainly by MAOs, are considered to be the decisive mechanisms of inactivation. The strong inhibition of the neuronal re-uptake of NA and 5-HT is the most prominent biochemical property of tricyclic antidepressants (Pletscher, 1973; Biel & Bopp, 1974; Iversen, 1975; Koe, 1975; Carlsson, 1976).

Nomifensine is a new antidepressant drug with a pharmacological profile somewhat different from that of the classical tricyclic antidepressants (Hoffmann, 1973; Gerhards *et al.*, 1974). Previous studies with synaptosomes have shown that the most relevant effect of nomifensine seems to be the strong inhibition of NA and DA re-uptake (Hunt *et al.*, 1974; Schacht & Heptner, 1974).

In the present study, various *in vitro* models (synaptosomes, electrically stimulated brain slices, and MAO preparations) have been used to obtain more precise information about the sites of action of nomifensine on brain monoamine metabolism, with special regard to the dissimilarities to tricyclic antidepressants as well as to psychostimulants such as amphetamine. As Costall *et al.* (1975) have suggested that nomifensine has a direct stimulatory component on DA receptors, we compared nomifensine with apomorphine (DA receptor stimulant) and clonidine (α-adrenoreceptor stimulant) in field-stimulated brain slices.

Furthermore, for the elucidation of a possible effect on 5-HT metabolism *in vivo*, the antagonistic potency of nomifensine on the depletion of brain 5-HT induced by fenfluramine, has been studied in comparison with chlorimipramine. Strong inhibitors of 5-HT up-take markedly antagonize the decrease in brain 5-HT induced by fenfluramine (Ghezzi *et al.*, 1973).

Methods

MAO activity

MAO activity was measured in mitochondrial suspensions prepared from rat liver and brain according to Chappel & Hansford (1969). After the final centrifugation step, the mitochondrial pellets were re-suspended in 67 mM K^+-Na^+- phosphate buffer, *p*H 7.0. Aliquots of the mitochondrial suspension were added to the reaction mixture containing K^+-Na^+-phosphate buffer (15 mM), *p*H 7.0, KCN (1 mM) and semicarbazide (10 mM). After

5 min pre-incubation at 37°C in the absence or presence of drugs, the reaction was started by adding tyramine hydrochloride as substrate (3.1 mM final concentration), and the enzyme activity was recorded by measuring the oxygen consumption polarographically in a Combi-Analysator (Eschweiler, Kiel), fitted with a stabilized all-glass platinum electrode, which is a modification of the Clark electrode (Gleichmann & Lübbers, 1960).

Enzyme activities are expressed in international units (1 IU = 1 μmol 0_2/min at 37°C) and are given as mean values ± s.d. of three determinations.

Uptake of biogenic amines by synaptosomes

^{14}C-NA (55 mCi/mmol), ^{14}C-5-HT (57 mCi/mmol) and ^{3}H-DA (2.3 Ci/mmol) were purchased from the Radiochemical Centre (Amersham). All reagents used were of analytical grade.

Synaptosomal fractions from rat brain were obtained according to the method of Whittaker (1969). Our methods for determining monoamine uptake were similar to those of Snyder & Coyle (1969) and have been described in detail elsewhere (Schacht & Heptner, 1974). Synaptosomal uptake of ^{14}C-NA and ^{14}C-5-HT was measured using Krebs–Henseleit bicarbonate buffer, *p*H 7.4, containing 11 mM glucose, whereas phosphate buffer was used for studying ^{3}H-DA uptake in synaptosomes from the corpus striatum (Kuczenski, 1975). Aliquots (2.5 ml) were taken from the synaptosome suspensions, and the samples incubated with labelled monoamine (1 x 10^{-7} M final concentration) at 37°C in a shaking water bath in the presence or absence of drugs. Incubation time was 5 min with synaptosomes from whole brain, 10 min with synaptosomes from hypothalamus, and 3 min with striatal synaptosomes. The reaction was terminated by cooling the tubes in ice. To determine non-specific adsorption, control samples were incubated at 0°C in otherwise identical conditions.

The amount of accumulated monoamine was evaluated using the membrane filtration technique (Hendley *et al.*, 1972), using a Millipore sampling manifold (Millipore GmbH, Neu-Isenburg) with cellulose nitrate filters, 25 mm in diameter and with 0.6 μm pore size (Sartorius GmbH, Göttingen). After collecting the synaptosomes under mild vacuum, the filters were transferred into counting vials and dissolved in 10 ml scintillation fluid. Radioactivity of the samples was determined in a Packard Tricarb liquid scintillation counter, and the amount of monoamine accumulated by the synaptosomes was expressed as percentage radioactivity added to the incubation mixture.

IC_{50} values were evaluated as the concentration of drug inhibiting the uptake of either ^{14}C-NA, ^{14}C-5-HT or ^{3}H-DA by 50%. For each drug, three or four concentrations were used at least in triplicate, and the results were plotted on semi-logarithmic paper.

The effects of nomifensine and D-amphetamine on newly accumulated catecholamines were investigated by measuring the uptake of ^{14}C-NA or ^{3}H-DA as a function of incubation time. Synaptosome suspensions obtained from rat hypothalamus were incubated for 10 min with ^{14}C-NA, then the drug was added, and the incubation was continued for another 10 min period. The amount of radioactivity remaining in the drug-treated synaptosomes after 20 min of total incubation time was calculated as a percentage of maximum ^{14}C-NA uptake into synaptosomes of the control samples run in parallel.

Drug effects on newly accumulated ^{3}H-DA were studied by adding the compounds after 6 min of pre-incubation and by pursuing the incubation for another 4-min period. Control samples were run in parallel for 10 min of total incubation time.

Electrical stimulation of brain slices

Our methods for investigating drug effects on the release of catecholamines from electrically stimulated brain slices were similar to those of Farnebo & Hamberger (1971). Round slices (diameter 2 mm, thickness 0.5 mm) of rat cerebral cortex and corpus striatum were prepared using the McIlwain Tissue Chopper (Mickle Laboratory, Gomshall) and a cylindrical punch. The cortical slices were incubated for 30 min at 37°C with 1 x 10^{-7} M L-[^{3}H]-NA (10.9 Ci/mmol) in a Krebs bicarbonate buffer, *p*H 7.4, and were continuously oxygenated by bubbling with 95% 0_2/5% CO_2. Incubation of striatal slices was carried out with 1 x 10^{-7} M ^{3}H-DA (2.7 Ci/mmol) in identical conditions.

After rinsing in fresh buffer for a few seconds, the slices were transferred to small stimulation chambers which we constructed from small plastic tubes. Six chambers were mounted in a rack and placed in a waterbath at 37°C (Figure 1). The slices were superfused with the oxygenated buffer at a rate of 0.5 ml/min in the absence or presence of drugs. After superfusion for 30 min, the tissue was stimulated by an electrical field characterized by rectangular biphasic pulses, which we monitored on an oscilloscope. Stimulation was carried out using pulses of 2 ms, with 10 Hz, and 11 mA for ^{3}H-NA-preloaded cortical slices and 14mA for ^{3}H-DA-preloaded striatal slices. The slices were stimulated for 2 min and further superfused for 18 minutes. The superfusate was collected in 5-min fractions for measurement of radioactivity.

In one group of experiments, the slices were stimulated twice for 2 min at 5 Hz with biphasic rectangular pulses of 11 mA and 2 ms duration, and with an interval of 30 min of recovery between the two stimulation periods.

At the end of the superfusion period, the radioactivity remaining in the slices was determined

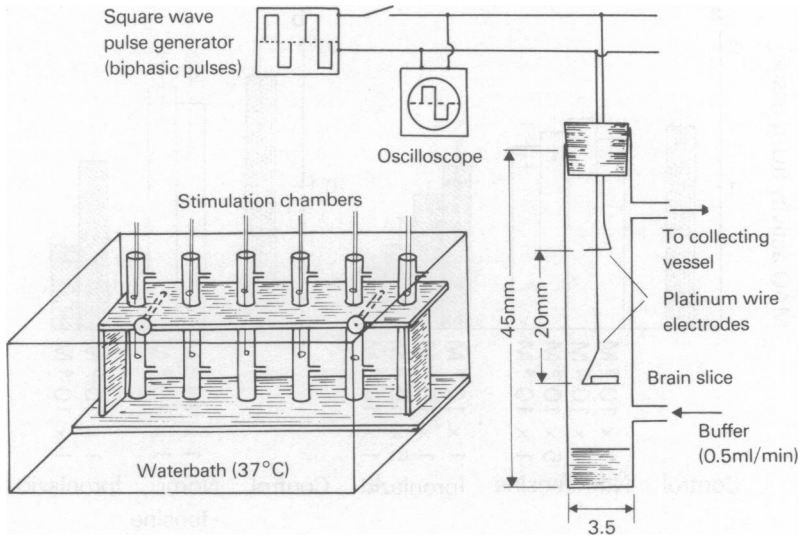

Figure 1 Apparatus for electrical stimulation of brain slices.

by catalytic combustion of the tissue at 700°C in an Oxymat JA 101 (Intertechnique, Mainz) and by liquid scintillation counting of the tritiated H_2O formed. The stimulation-provoked overflow of radioactivity was calculated by subtracting the estimated spontaneous ^3H efflux from the total ^3H efflux during the same period, and is expressed as a percentage of the ^3H content in the slice at the onset of stimulation (Farnebo & Hamberger, 1971a). The drug-induced changes in the overflow of catecholamines from the slices are presented as a percentage of drug-free controls. The results are given as mean values ±s.d. (n = number of slices).

Fenfluramine-induced 5-HT depletion in the brain

Treatment was carried out according to Ghezzi *et al.* (1973). Female Wistar rats weighing 150–200 g were used. Two doses of nomifensine (10 and 30 mg/kg i.p.) or chlorimipramine (10 and 20 mg/kg i.p.) were administered to the animals. After 30 min, the rats received fenfluramine 15 mg/kg i.p. and were killed 2 h later for the biochemical assay of 5-HT in the brain. The controls were injected twice with NaCl solution. 5-HT was determined fluorometrically using the method of Miller *et al.* (1970).

Results and Discussion

In vitro studies with MAO preparations obtained from rat liver and brain have demonstrated that nomifensine up to 1 x 10^{-4}M does not inhibit MAO, whereas iproniazid induces a marked and concentration-dependent decrease in the activity of both liver and brain enzyme (Figure 2). After measuring MAO in liver, heart and brain of rats after administration of nomifensine 10 mg/kg i.v. or 20 mg/kg p.o., no alteration in the enzyme activity was found in the drug-treated animals (Hoffmann & Schacht, unpublished). Thus, we may exclude an essential role of nomifensine as a MAO inhibitor.

Synaptosomes (pinched-off nerve endings) can serve as an excellent *in vitro* model for studying drug effects on the uptake process of biogenic amines. These subcellular particles exhibit the biochemical and morphological characteristics of nerve endings *in situ*, and they are capable of incorporating neurotransmitters from an incubation medium by a carrier-mediated high affinity uptake process (Whittaker, 1969; Whittaker & Barker, 1972; Iversen, 1975; Jones, 1975).

Accumulation of labelled NA in a crude synaptosome preparation from rat brain occurs almost linearly during the initial 5 min of incubation and reaches a plateau after about 30 min of incubation (Figure 3). Regional differences in the uptake of biogenic amines in synaptosomes isolated from different brain areas, and the inhibitory activity of a large number of compounds towards the various monoamines, have been studied thoroughly by many investigators (Snyder & Coyle, 1969; Horn *et al.*, 1971; Kannengiesser *et al.*, 1973; Kuhar, 1973; Ross & Renyi, 1975).

Most of the tricyclic antidepressants block the uptake of NA and 5-HT, but they differ with respect to relative potency in these systems. In general, the tertiary amines among these drugs (for example,

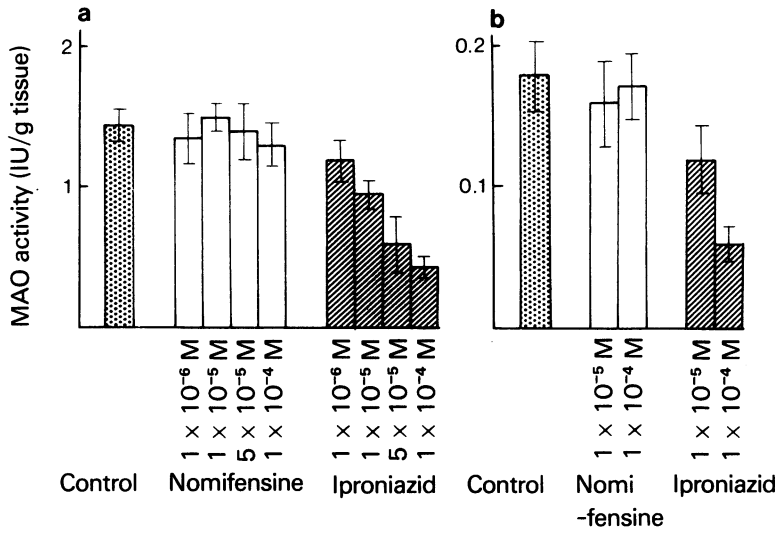

Figure 2 Inhibition of MAO *in vitro*. a, Liver; b, brain.

chlorimipramine and imipramine) reveal a strong inhibition of 5-HT uptake, whereas the secondary amines (for example, desipramine) inhibit more strongly the NA uptake in synaptosomes from hypothalamus, used as a model of noradrenergic nerve terminals. These results, together with clinical observations, have given rise to the suggestion that 5-HT is primarily involved in the control of mood, and NA in psychomotor activity (for refs, see Carlsson, 1976).

The tricyclic antidepressants exert only weak inhibitory activity on DA uptake in striatal synaptosomes (Horn *et al.*, 1971). Nomifensine is almost as potent as desipramine in blocking NA accumulation in hypothalamic synaptosomes (Table 1). Moreover, in contrast to the tricyclics, it strongly inhibits the NA uptake in whole brain synaptosomes as well as the DA uptake in striatal synaptosomes (Hunt *et al.*, 1974; Schacht & Heptner, 1974). Rather

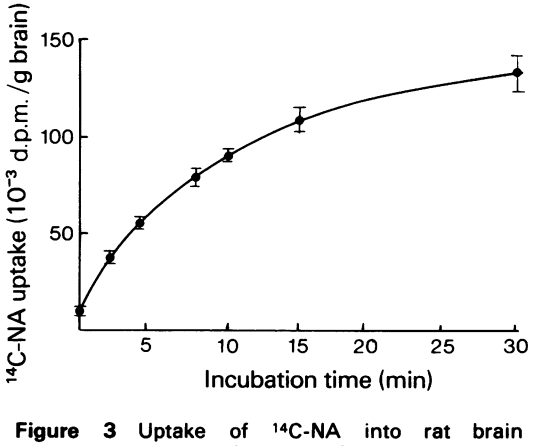

Figure 3 Uptake of ^{14}C-NA into rat brain synaptosomes as a function of incubation time. Characteristics of neurotransmitter uptake into synaptosomes: carrier-mediated high affinity process; temperature dependent; ion concentration dependent; energy dependent (Na^+-K^+-ATPase); saturable process (Michaelis-Menten kinetics).

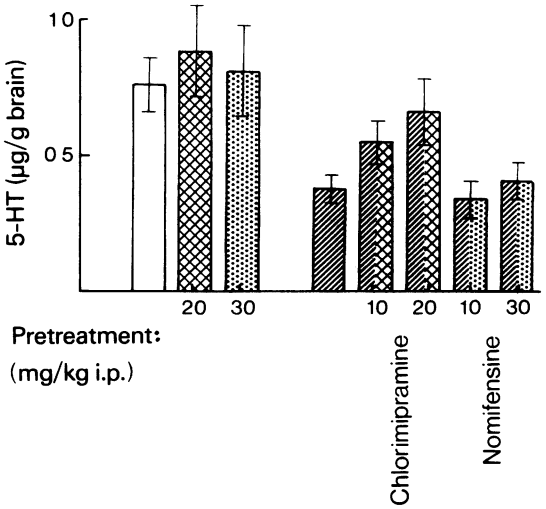

Figure 4 Effect of chorimipramine (cross-hatched columns) and nomifensine (dotted) on fenfluramine-induced (hatched) (15 mg/kg i.p.) depletion of 5-HT in rat brain. Open column, Control.

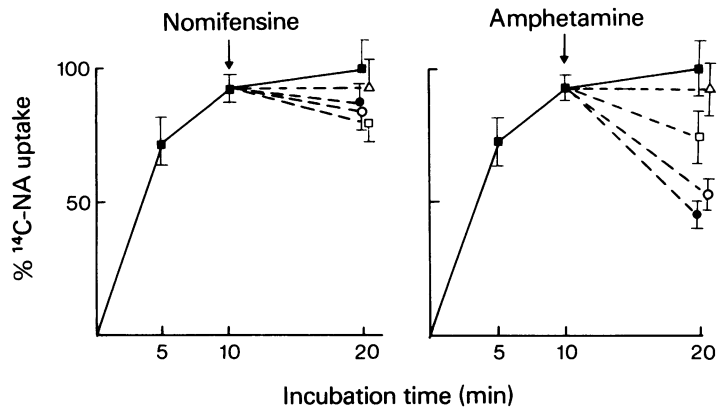

Figure 5 Uptake of ^{14}C-NA into rat hypothalamus synaptosomes and effects of drug addition on the accumulated ^{14}C-NA. Concentrations (M): ■, control; △, 1×10^{-7}; □, 1×10^{-6}; ○, 1×10^{-5}; ●, 1×10^{-4}.

high concentrations of nomifensine are required for the blockage of 5-HT uptake.

Biochemical studies *in vivo* on the counteraction of nomifensine against monoamine-depleting agents like fenfluramine and 6-hydroxydopamine (6-OHDA) confirm the pattern of inhibitory activity on monoamine accumulation found *in vitro*. The decrease of 5-HT in rat brain induced by fenfluramine is antagonized by chlorimipramine but not by nomifensine (Figure 4). Fenfluramine produces a long-lasting depletion of brain 5-HT by promoting the release of 5-HT from its storage sites (Fuxe *et al.*, 1975; Kannengiesser *et al.*, 1976). Since the 5-HT-depleting action of fenfluramine is antagonized by pretreatment with strong 5-HT uptake blockers, like chlorimipramine (Ghezzi *et al.*, 1973), fenfluramine probably uses the membrane-bound amine uptake system ('membrane pump') to enter the 5-HT neurones. The lack of efficacy of nomifensine in counteracting the effect of fenfluramine *in vivo* indicates that the new antidepressant does not exert a significant effect on 5-HT re-uptake in the brain. Therefore, as nomifensine has also been reported to reveal mood-elevating activity, the above mentioned hypothesis suggesting a correlation between blockage of 5-HT re-uptake and elevation of mood still remains questionable.

On the other hand, nomifensine strongly antagonizes the decrease in brain DA and NA induced by an intraventricular injection of 6-OHDA (Samanin *et al.*, 1975). As the catecholamine-depleting action of 6-OHDA has been reported to be dependent on its uptake, the antagonizing effect of nomifensine may be attributed to blockage of uptake of 6-OHDA into noradrenergic and dopaminergic neurones. Samanin *et al.* (1975) found desipramine to be ineffective against the depletion of DA induced by 6-OHDA, thus confirming the dissimilarities between nomifensine and the tricyclic antidepressants with regard to the different activities on DA uptake established in synaptosomes.

Although nomifensine fails to provoke phenelzine potentiation in mice (Kruse *et al.*, 1977), in agreement with its weak activity in blocking 5-HT uptake, a certain influence on serotoninergic function cannot be completely excluded. Maj *et al.* (1976) have found an increase in 5-hydroxyindole acetic acid (5-HIAA), the main metabolite of 5-HT, in rat brain 4 h after a single injection of nomifensine 20 mg/kg i.p. The brain level of 5-HT itself was found to be elevated 2 h after administration of nomifensine 40 mg/kg. These effects of nomifensine, indicating enhanced 5-HT turnover, may be related either to an interaction between the various monoaminergic systems, most

Table 1 Inhibition of monoamine uptake into rat brain synaptosomes

Antidepressant	IC_{50} (M)			
	^{14}C-NA (Whole brain)	*^{14}C-NA (Hypothalamus)*	*^{3}H-DA (Corpus striatum)*	*^{14}C-5-HT (Whole brain)*
Nomifensine	3.5×10^{-8}	3.2×10^{-8}	1.4×10^{-7}	1.2×10^{-5}
Imipramine	2.2×10^{-5}	0.8×10^{-7}	3.4×10^{-5}	3.5×10^{-7}
Desipramine	1.8×10^{-5}	2.5×10^{-8}	2.0×10^{-5}	1.1×10^{-5}
Chlorimipramine	1.6×10^{-5}	6.4×10^{-7}	1.2×10^{-5}	2.0×10^{-8}

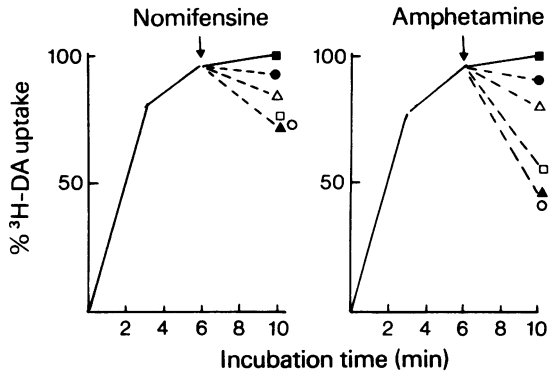

Figure 6 Uptake of ^{3}H-DA into rat striatum synaptosomes and effects of drug addition on the accumulated ^{3}H-DA. Concentrations (M): ■, control; ●, $3x10^{-8}$; △, $1x10^{-7}$; □, $1x10^{-6}$; ▲, $3x10^{-6}$; ○, $1x10^{-5}$.

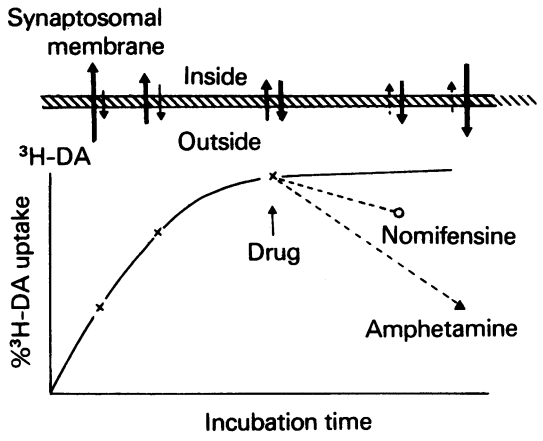

Figure 7 Scheme of relative velocities of DA uptake and release *in vitro*.

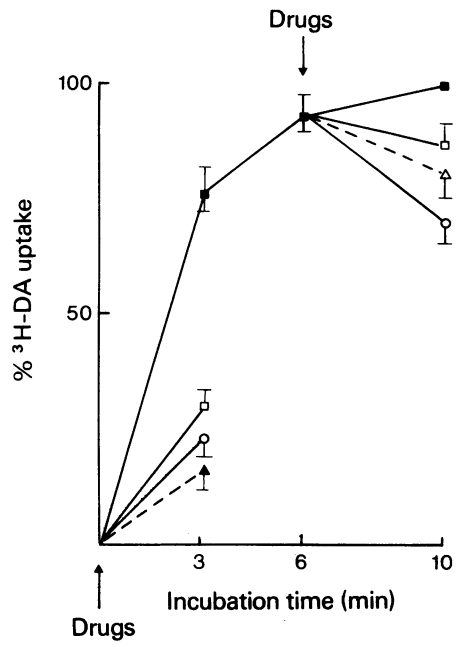

Figure 8 Uptake of ^{3}H-DA into rat striatum synaptosomes: different effects of drugs added at different times. Concentrations (M): ■, control; □, nomifensine $1.0x10^{-7}$; ○, amphetamine $3.5x10^{-7}$; ▲, nomifensine $1.0x10^{-7}$ and amphetamine $3.5x10^{-7}$.

probably mediated by the increase in central dopaminergic function; or they may be due to an influence on serum tryptophan binding, which seems to be an important factor in regulating 5-HT biosynthesis in the brain (for refs, see Wurtman & Fernstrom, 1976).

Although nomifensine seems to share its action on the dopaminergic system with amphetamine, its mechanism of action is clearly different from that of the psychostimulant drugs. Amphetamine and its derivatives, in addition to their inhibitory activity on monoamine uptake, are acting mainly by promoting the release of catecholamines into the synaptic cleft (for refs, see Estler, 1975). The effects of nomifensine, however, on the catecholamine system, including the small increase in striatal DA turnover (Gerhards *et al.*, 1974), are not caused by an enhancement of catecholamine release.

Figure 5 shows the influence of nomifensine and D-amphetamine on newly accumulated NA in hypothalamic synaptosomes. Drug addition after 10 min of incubation, when the steady state of NA accumulation has almost been attained, induced a decline in intrasynaptosomal ^{14}C-NA evaluated after 10 min of further incubation. Subsequent to the addition of high concentrations of nomifensine, total NA uptake was found to be about 20% less than in the controls at 20 min of incubation, and no concentration-dependent potentiation of activity was achieved. In contrast, with amphetamine 10^{-4} M the decrease in incorporated NA amounted to 54% of the control, this effect being based on an enhanced release of labelled NA accumulated during the initial 10 min of incubation.

Studying drug effects on DA uptake in striatal synaptosomes as a function of incubation time, the same fundamental difference between nomifensine and amphetamine has been obtained (Figure 6). Indeed, after addition of high concentrations of nomifensine there is a slightly more rapid decline in the accumulation of DA than in that of NA. Again, these effects are induced by the strong inhibition of DA uptake and are not due to an enhanced release of DA. The schematic graph in Figure 7 illustrates the dissimilar effects of drugs on the DA uptake. The

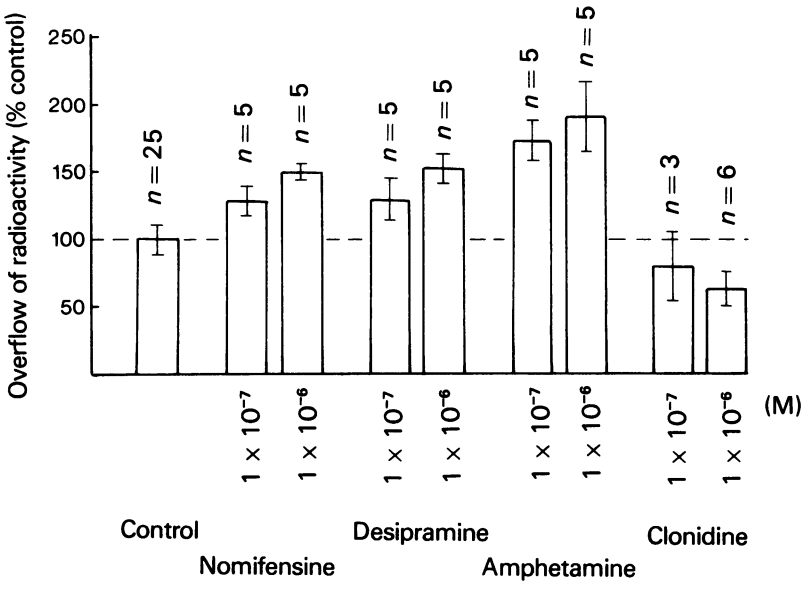

Figure 9 Drug-induced changes in the overflow of ^{3}H-NA from field-stimulated cerebral cortex slices. Electrical stimulation for 2 min (2 ms, 11 mA, 10 Hz). Mean ± s.d.

relative velocities of DA movement across the synaptosomal membrane as a function of incubation time, as well as of sites of drug action, are symbolized by arrows. Reaching the plateau means that a steady state has been reached in which the rates of uptake and spontaneous release have become equal, and that these processes are occurring much more rapidly with DA than with NA. Consequently, the complete blockage of DA uptake by high concentrations of nomifensine induced a small but significant decrease in the amount of intrasynaptosomal labelled DA, as the spontaneous release of the monoamine continued. After addition of the same concentrations of amphetamine, the decrease was more pronounced, since amphetamine, beyond its activity in blocking the uptake process, enhanced the DA release.

The postulate of dissimilar sites of action for nomifensine and amphetamine is strongly supported by establishing different effects of the drugs added at different times during the incubation period (Figure 8). A mixture of nomifensine and amphetamine has been used, the concentrations being in the range of the respective IC_{50}. The addition of the mixture, after 6 min of pre-incubation with labelled DA only, caused a less marked decrease in incorporated DA than the same concentration of amphetamine alone, indicating that nomifensine antagonizes the effect of amphetamine on DA release. Amphetamine probably has to enter the synaptosomes, utilizing the catecholamine uptake system, before it can act on the storage sites from which the catecholamines are

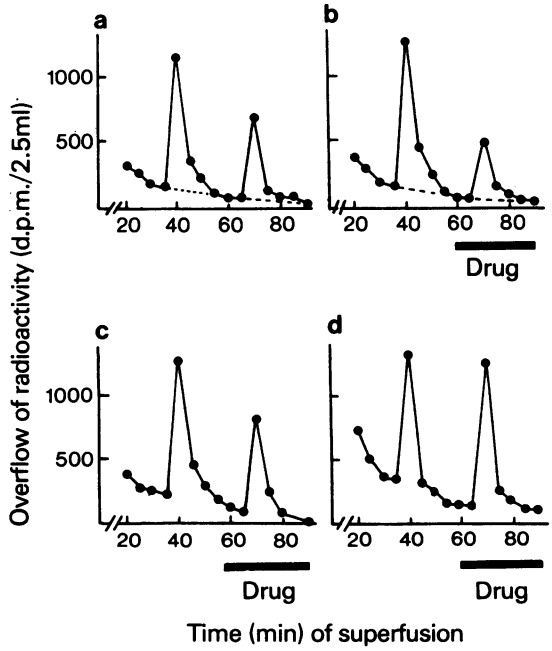

Figure 10 ^{3}H-NA overflow into the buffer during superfusion and electrical stimulation of individual cerebral slices. Drug effects during the second stimulation. Concentrations (M); a, control; b, clonidine $1x10^{-6}$; c, nomifensine $1x10^{-6}$; d, (+)-amphetamine $1x10^{-7}$.

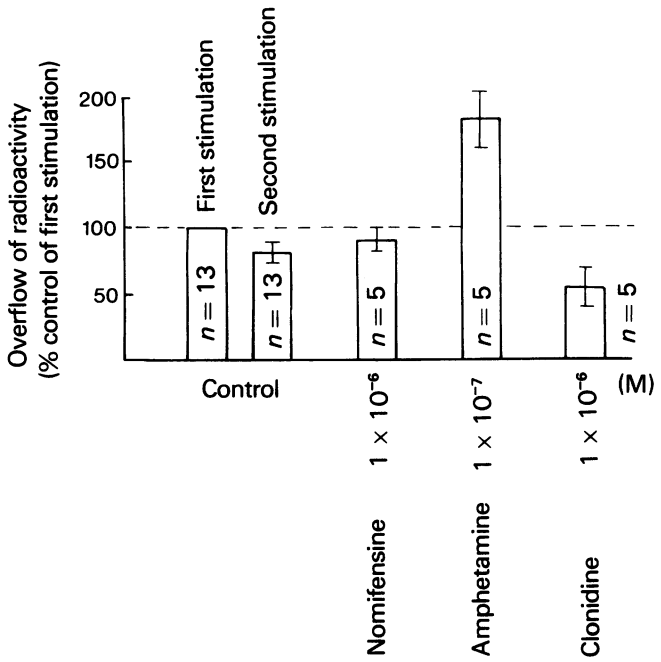

Figure 11 Field-stimulated cerebral cortex slices: drug-induced changes in the overflow of ^{3}H-NA during the second stimulation period. Electrical stimulation for 2 min (2ms, 11 mA, 5 Hz). Mean ± s.d.

released. Nomifensine seems to block the uptake of amphetamine, because of its strong activity on the membrane pump. When the mixture of both drugs was added simultaneously with labelled DA—that is, at zero time of incubation—the drug effects were additive with regard to the diminished DA uptake.

Stimulation of isolated and superfused brain slices in an electrical field (Figure 1) has proved to be an adequate model for studies on monoamine release in the brain (Farnebo & Hamberger, 1973; Starke & Endo, 1976). Release–that is, the actual output of transmitter into the extracellular space elicited by nerve stimulation—cannot be measured directly in these experiments. Only the fraction that escapes re-uptake and metabolism, and consequently diffuses into the superfusion fluid, can be experimentally determined. The term 'overflow' is commonly used for this fraction.

When one or several sites of loss (neuronal and extraneuronal uptake, metabolizing enzymes) are inhibited, an increase in monoamine overflow will be obtained which is not due to changes in the amount of monoamine released by nerve stimulation. An increase in overflow can also be achieved when the actual release of the transmitter is enhanced, even if the sites of loss are not affected. A decrease in the release can be elicited by drugs interfering with the

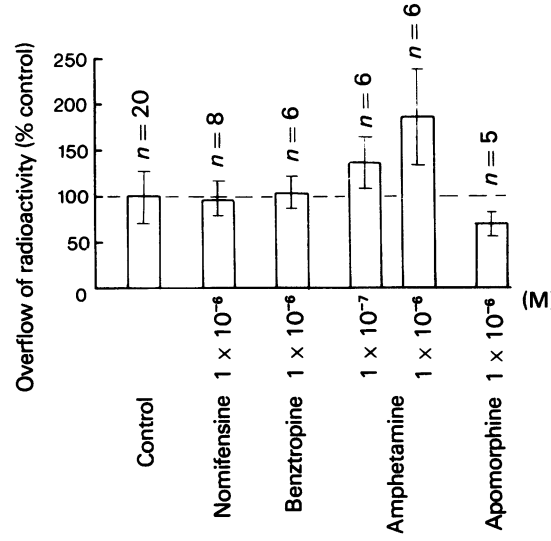

Figure 12 Drug-induced changes in the overflow of ^{3}H-DA from field-stimulated slices of rat corpus striatum. Electrical stimulation for 2 min (2 ms, 14 mA, 10 Hz). Mean ± s.d.

mechanisms that regulate transmitter output (Langer, 1973).

When cerebral cortex slices pre-loaded with labelled NA were superfused with nomifensine, an increases in the field-stimulated overflow of 30% and 51%, respectively, have been measured, which correspond exactly to those obtained with desipramine in the same concentrations (Figure 9). These changes refer to the strong blockage of NA re-uptake by both antidepressants. On the contrary, amphetamine, even at 10^{-7} M, increased the overflow by 73%. Since amphetamine exerts a less potent activity on NA uptake, the stronger increase in overflow reflects the enhanced release. Clonidine, an α-receptor agonist with a powerful antihypertensive action, decreases the overflow of NA. This effect is interpreted by the working hypothesis of release-modulating α-receptors located presynaptically. Activation of these presynaptic α-receptors reduces the release of transmitter per nerve impulse (for refs, see Starke & Endo, 1976).

The more quantitative difference between nomifensine and amphetamine, as shown in Figure 9, can be changed into a qualitative one by modifying the experimental conditions in such a manner, that electrical stimulation of the slices with lower frequencies is carried out twice. Using this procedure, the slices were superfused with the drug-free buffer during the first stimulation period and with the drug-containing medium during the second stimulation period, as illustrated in Figure 10. Thus, the overflow provoked by the first stimulation served as internal control for the overflow produced by the second stimulation of the respective slice. In control experiments, when the slices were superfused without any drug during the total period, the overflow of NA elicited by the second stimulation amounted to a mean of 80% of that induced by the first stimulation (Figure 11). It should be mentioned that the absolute amount of overflow of radioactivity is not indicated by the peak height of the diagrams shown in Figure 10, but is given by the area circumscribed by the straight and dotted lines.

The results of a series of experiments with a twofold stimulation of slices are summarized in Figure 11. Clearcut dissimilarities in the action of nomifensine, amphetamine and clonidine are demonstrated in this model.

By measuring the changes in the overflow of labelled DA from field-stimulated striatal slices in the presence of nomifensine, the lack of any releasing activity was confirmed (Figure 12). Nomifensine and benztropine did not change the overflow, whereas amphetamine induced a marked increase. Since apomorphine, a DA receptor agonist, reduced the overflow, we may conclude that nomifensine does not stimulate DA receptors directly, but exerts its dopaminergic activity by an indirect mechanism—that is, by inhibiting DA re-uptake.

Conclusions

Nomifensine potentiates neurotransmission in both noradrenergic and dopaminergic synapses, based on its strong potency in blocking catecholamine re-uptake. According to the results presented, nomifensine does not fit into any of the known groups of antidepressant drugs. It exhibits the properties of a new type of antidepressant. Nomifensine does not inhibit MAO and does not enhance the release of biogenic amines. From the studies in electrically stimulated brain slices, we also may exclude a direct stimulating activity on DA and NA receptor sites. 5-HT uptake is not inhibited by concentrations relevant to the therapeutic dosage of nomifensine. The mechanism of increasing the turnover of 5-HT in rat brain remains to be elucidated. Nomifensine differs from the tricyclic antidepressants by its additional effect on the dopaminergic system; and this might explain some of the results which have been obtained in studies on animal behaviour and which are not observed with the tricyclic antidepressants. We suggest that the potentiation of dopaminergic function is jointly responsible for the therapeutic activity. This suggestion has been supported by clinical investigations demonstrating some evidence for a disturbance in DA metabolism in patients suffering from endogenous depression (Birkmayer & Riederer, 1975; van Praag & Korf, 1975).

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Discussion

DR WOODRUFF (Southampton) asked whether the effect of nomifensine on sympathomimetic amines such as oxymetazoline or methoxamine had been studied, as such a study could indicate the effect of nomifensine on postsynaptic receptor sensitivity.

DR SCHACHT (Frankfurt) replied that such a study had not been carried out. He added that nomifensine did not increase the motility of rats pretreated with reserpine, indicating that its action depended on catecholamines.

PROFESSOR TURNER (Chairman) asked whether Professor Spencer could relate his interpretation of the monoamine theory, and acute pharmacological effect of drugs, to their long-term clinical antidepressive effects.

PROFESSOR SPENCER (Cardiff) agreed that there were difficulties in relating clinical improvement and pharmacological activity. He suggested that the relationship of changes in cyclic AMP and metabolites to clinical improvement should be examined more closely.

DR SHAW (Cardiff) pointed out the increasing difficulty of drawing recent clinicopharmacological data into a single hypothesis. He sited, for example, the inverted U plasma level response curve, which characterizes the secondary amine tricyclics, and the straight line plasma level response curve of at least two tertiary amines. Secondly, he sited mainserin, a 5-HT blocker, which also possesses antidepressive activity.

PROFESSOR SPENCER said that the discussion highlighted the problems facing all those working in this area, whether with animals or patients.

DR ST. JOHN (Lincoln) asked what was the effect of overdosage of nomifensine.

DR HOFFMANN (Frankfurt) replied that at very high dosage, nomifensine provoked convulsions followed by death from respiratory failure.

DR HANKS (London) reported a clinical case of overdosage with 30 capsules, a total of 750 mg of nomifensine. The patient recovered, without adverse sequelae. In particular, no signs of cardiovascular toxicity were seen.

DR HOFFMANN reported that diazepam could reverse some of the overdosage effects of nomifensine.

In reply to a question whether there was any evidence suggesting the development of dependence on nomifensine, PROFESSOR VOGEL (Frankfurt) reported that in formal studies in primates dependent on amphetamine, there was no substitution by nomifensine. Nor was there substitution in monkeys dependent on cocaine.

PROFESSOR TURNER asked whether anorectic activity had been noted.

DR HOFFMANN replied that nomifensine possessed no anorectic activity in monkeys.

DR LLOYD (Bridgend) attributed the delay in onset of antidepressive activity in patients treated with imipramine to inappropriate dosage. In his view, initial dosage of imipramine should be as high as 400–500 mg daily. Used in this manner, antidepressive activity could be observed within 48 hours.

In this context PROFESSOR TURNER raised the problem of patient compliance. Between 30–40% of all patients participating in a current antidepressive drug study had no detectable levels of drug present in their plasma. Even patients known to be taking their medication revealed 20- 30- or 40-fold differences in plasma levels for a given number of tablets taken. At least some of the reasons for variation in response, he suggested, were beyond the psychiatrists' control.